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Title: IMMUNOGENIC PILI
PRESENTING FOREIGN PEPTIDES,
THEIR PRODUCTION AND USE
Inventor(s): Peter O'Hanley et al.

DOCKET NO.: 050939/0104

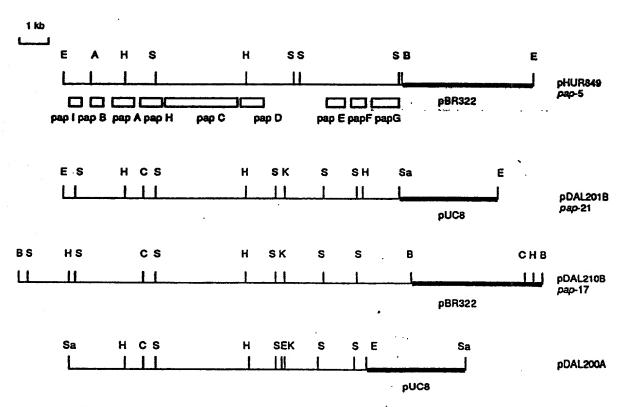


Figure 1. Genetic and physical map of recombinant plasmids pHUR849, pDAL201B, pDAL210B, and pDAL200A. The locations of of the pap genes shown as open bars. Restriction site abbreviations: A, Apa I; B, Bam HI; C, Cla I; E, Eco RI; K, Kpn I; Sa, Sal I; and S, Sma I.

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•	A R L R F S V P L F F G C V F V H G V -22		ATMANCTOCOATTCTCTTTTTTTTTTTTTTTTTTTTTTTTTTT	3
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	V W W D G R A A F R G E V V R P A C T L	180	CHATGATGAGAGAGAGAGATTCATGAGAGATTCAGAGCTGCCTGACACTGA	780
=	GCGATGGALGAGGCCTGGCAGATTATTGATATGGGGGAAAACCCGGGTAGGAATTACAGAAAAACCCGGGGAGAATTACAGAAAAACCCGGGTAGAAAAGCCGGGAAAAAGCGGGAAAAAGCGGGAAAAAGAAAAAA	240	OCCATOGNAGACCTTCCCASTTATCGATATACCCAAAACCCCCGGTTCCGGATTTACACAA WAAAAAAAAAAAAAAAAAAAAAAAAAAAA	350
2	ATTGSTITCTCCCACCTCAAAAAAAATTCAGCCTCCCCCTCACCAATTGTCAAATTAAACAAAAAAAA	300 241	ATTUGATATCTCOGACCTGAAAAATTVACCTCCOOCTCAGAAACTTAAC	300
301	AGTEAGGGGAACTITYCTCAATTCCCGGATAAGGGGAACTITCGAAGGGGGGCGGGGGGGGGG	360	AGTUAGGSTGGAAACCTITITCTCTGATTCCCGGAAAAACGGTCACTGGGGGGGGGG	360
36	GOTGANAGCOGGANAGTTANTTATACGGACAGGCANAGGANTANTCTGCAGANAGG B T P D K F N L S G Q A K G I N L G I	420 361	GTGANACCCGATAAGTITAATCCGGTCAGCCAAAGGAATTAATCTGCAATTA	420
5 ,	CCTGATOTOROGOGIANTATIC <u>CCGGGCAGGAANOTANTGCCTGCATTGAGG</u> A D V R G N I A R A G K V N P A I P L T	480 421	A D A R G N I A R A G K V N P A, I P L T	9
\$	GGTATGAGAACCCTGATACACCCTCAGAATTGTGAGAACAAAAAAAA	540 481	GOTANTAMAAAGGGTDAATTACADCTTAAAATTOTGGAAAAGGAAAAAACTTAAA G N B B A L D Y T L R I V R N G K K L E	\$6
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,	Anchartocanity contribution in the contribution of the contributio		ATGACACTCCCATTCTCTTTTTTTTCTGTGTGTTTTTTTT	8
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171	OTHITOTOGRAPOCHOCTOTITICATOGRAPOTATOCACCTOCACTOTACTCTC V W W D G R A A F H G B V V R P A C T L	190 121	-1.4.1 GINTOGRACOGCACOGCTITTCARGOTAGACGTTOTCACACCTGCCTGTACTCTG V W W D G R A A P H G E V V R P A C T L	180
5 .	OCHARGAMCACCCTOCCHCATATICAZÁTOCCCCCATATCCCCATATACCACATATACCACACACACA	240 . 101	OCCANTOGANCA COCCUCACA TANDA CANTOCACA A MEDAN WE IN IN MER TO WE IN	
2	ATTOCKT TITCOGACCT GAAGAAAT CACCTCCGGCTCAGAACTGTGAATTAAC	300 241	ATTOSTITITICCOCACCICANAAAAAAAATTCACCTCCOCCTCACAACTTTAACAA GAAAAAAAAAAAAAAAAAAAAA	300
301	AGREAGASTICACATIVETETRATECEGGATAAGGTAACTITEGATGGCGTCCGG	360 301	ACTCACOCTOCCAACCTTTTCTCTCAATACCCCCAATAACCTCACCAACCTTTTCCATCCTCC	360
361	GOTCHANGCCGGATHAGTTHATCGGTCHGGCAAAAGCCATTAATCTGCAGATH G E T P D K F N L S G Q A K G I N L Q I	420 361	GOTGANACCCCCCATANACTTANTCCCCTCACCCANACCCATTANTCTCCCACATA	620
123	OCTCATOCCACOCIAATIOCCOCACACACATAACATAACA	480 421	GCTGATGCCAGGGGAAATATTGCCTGCAATACGTTGACGAA D A R G N I A R A G K V M P A I P L T	0
3	GOTATUMANOCOCICANTICACCOCICAMATICAMANOCICAMANACTICAMA O N E E A L D Y T L R I V R N G K K L E	540	GOTATION CANCECTE CATTACCE CLOCATION COLOR MAN COLOR LE E O Y T L R I V R N G K K L E	. 540
3	A G N Y F A V L G F X V D Y E .	541	OCCOGANATATTTCCCTCCTCCATTCCCCCTCGATTATCACTCA A G M Y F A V L G F R V D Y B C	

Figure 2. DNA sequences of pHUR849 (A), pDAL201B (B), pDAL210B (C), and pDAL200A (D) papH structural genea. The nontranscribed DNA strand for each close shown. Numbering is from the 5' end . The deduced amino acid sequence for the correct frame is shown below each DNA strand. The first amino acid of the mature protein is +1. Stop codon is marked with asterisks. The restriction sites for Cla! (ATGGTA), and Sma! (CCCGGG), are underlined once, respectively.

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		•		
	1			60
pHUR849	ATGAGACTGCGATTCTC	TGTTCCACTTTTCTTTTTTGG	CTGTGTGTTTGTTCATGG	ICTT
pDAL200A		· •	•	
pDAL201B		t		
pDAL210B		t		
•	61 67			120
pHUR849	TTTGCCGGTCCGTTTCC	TCCGCCCGGCATGTCCCTTCC	TGAATACTGGGGAGAAGA	GCAC
pDAL200A		t		a.
pDAL201B		t		a
pDAL210B				a
	121			180
pHUR849	GTATGGTGGGACGGCAG	GGCTGCTTTTCATGGTGAGGT	TGTCAGACCTGCCTGTAC	_
DDAL200A				
pDAL201B				
DAL210B				
,	.181			240
pHUR849		GCAGATTATTGATATGGGGGA	A A COCCOCCUE ACCCCA THERM	
pDAL200A	acou toouroucocc to	C CONTRACTOR THE CONTRACTOR CONTR	L E	aunu
pDAL201B		-	•	
DOAL210B		C .	t .	
PUNCETUB	242	c	t	300
pHUR849	241			300
	ATTGGTTTCTCCGGACC	TGAAAGAAAATTCAGCCTCCC		TAAC
pDAL200A			c	
pDAL201B pDAL210B	£		e e	
PONTSION	t		· c	
pHUR849	301			360
pDAL200A	AGTEAGGGTGGGAACET	PTTTCTCTGATTCCCGGATAAC	GGTGACTTTCGATGGCGT	CCGG
pDAL2018				
oDAL210B		•	_	
PUNLZIVE			t	
pHUR849	361	4		420
	GGTGAAACGCCGGATAA	GTTTAATTTATCCGGTCAGG		GATA
pDAL200A		•	a	
pDAL201B				
pDAL210B				
-11110040	421			480
pHUR849		NTATTGCCCGGGCAGGAAAAG 1	AATGCCTGCAATACCAT1	GACG
pDAL200A	c	g		
pDAL201B	c	g		
pDAL210B	c	g		
	481			540
pHUR849	GGTAATGAAGAAGCGCT	rggattacaccctcagaattgt	Cagaaacggaaaaaaact	TGAA
pDAL200A			C	
pDAL2018			c	
pDAL210B			c	
	541	• •	588	
pHUR849	GCCGGAAATTATTTTG	etgtgctgggattccgggtcg/	TTATGAGTGA	
pDAL200A		c		
pDAL201B		c		
pDAL210B		c ·		

Figure. 3. Comparison of deduced nucleotide sequence of papH genes pHUR849, pDAL200A, pDAL201B and, pDAL210B. The nucleotide(nt) identities, compared with the deduced sequence of PapH nt sequence papH gene of pHUR849 (upper case), are indicated by blank space, nt differences for the PapH genes of pDAL201B, pDAL210B and, pDAL200A, are shown with the corresponding single letter nt code (lower case), respectively. Numbering is from the 5' end. The first nt of coding for the leader sequence is numbered 1, and the first nt coding for the mature protein is numbered 67.

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-1 +1 18

MRLRFSVPLFFFGCVFVHGVFA¹GPFPPGMSLPEYWGEEH

19

VWWDGRAAFHGEVVRPACTLAMEDAWQIIDMGETPVRDLQ
59

NGFSGPERKFSLRLRNCEFNSQGGNLFSDSRIRVTFDGVR
138

GETPDKFNLSGQAKGINLQIADVRGNIARAGKVMPAIPLT

173

GNEEALDYTLRIVRNGKKLEAGNYFAVLGFRVDYE

Figure. 4. Comparison of deduced amino acid sequence of papH genes pHUR849, pDAL201B, pDAL210B and, pDAL200A. The vertical arrow indicates the postulated cleavage site for the signal peptidase. The amino acid (aa) identities, compared with the deduced sequence of PapH protein of pHUR849 (upper case), are indicated by blank space, as differences for the PapH proteins of pDAL201B, pDAL210B and pDAL200A, are shown with the corresponding single letter as code(lower case), respectively. The first as of the leader sequence is numbered -22, and the first as of the mature protein is numbered +1.

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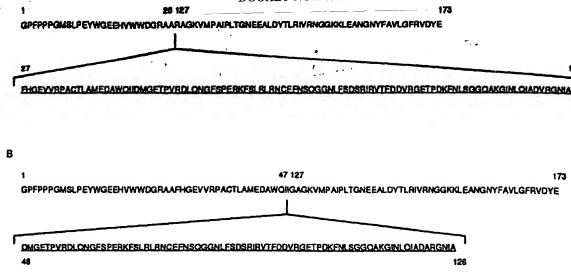


Figure. 5. Comparison of deduced amino acid sequence of papH gene deletion mutants pHUR849-5(pap-5), pDAL201B(pap-21), pDAL210B(pap-17) and, pDAL200A(pap 200A). The deduced amino acid sequence of each of the finial constructs is shown, (A) pHUR849-5 and, (B) pDAL201B, pDAL210B and, pDAL200A which are identical to each other. The amino acid identities of the proteins are upper case letters. The first amino acid of the mature fusion protein is numbered 1. The underlined sequence indicates the amino acid residues deleted from the mature fusion protein of each strain.

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